REMARKS

Claims 1-20 and 58-79 are pending and under examination. Claims 1, 20, 59 and 78 have been amended. New claims 80-99 have been added. Support for the amendments and new claims can be found throughout the specification and the claims as filed. In particular, support for the amendment to claims 1 and 59 can be found, for example, on page 30, lines 6-9. Claims 20 and 78 have been amended to clarify antecedent basis. Support for new claims 80-99 can be found, for example, in original claims 1-20 and on page 30, lines 6-9. Accordingly, these amendments and new claims do not raise an issue of new matter and entry thereof is respectfully requested.

Regarding the Priority Claim

Regarding the priority claim, Applicants respectfully maintain the position of record that parent provisional application serial No. 60/209,539, filed June 5, 2000, provides sufficient description and guidance to enable the claimed methods. Accordingly, priority should be granted to the June 5, 2000, filing date of parent application 60/209,539.

Rejection Under 35 U.S.C. § 112, First Paragraph

The rejection of claims 1-20 and 58-79 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement is respectfully traversed. Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods.

Applicants respectfully maintain, for the reasons of record, that the specification provides sufficient description and guidance for the claimed methods of differentiating cells to produce a cell population containing neuronal cells protected from apoptotic cell death both *in vivo* (claims 1-20 and 58) and *in vitro* (claims 59-79). Applicants further maintain the position of record regarding the references asserted to support a lack of enablement. With respect to Rossi and Cattaneo, Nat. Rev. Neurosci. 3:401-409 (2002), Applicants maintain that any issues that may exist for generating homogeneous populations of neural stem cells or the functional integration of donor neural stem cells and their biological properties as discussed in Rossi and Caettaneo are not relevant to the claimed methods, which specifically recite differentiating cells to produce a cell population containing neuronal cells protected from apoptotic cell death.

Regarding the Cao et al. reference, J. Neurosci. Res. 68:501-510 (2002), Applicants maintain that this reference describes the manipulation of endogenous neural precursors as challenging and unsuccessful. In contrast, the claimed methods are directed to differentiating progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death by contacting the progenitor cells with a differentiating agent and introducing into the progenitor cells a nucleic acid encoding an active MEF2 polypeptide or active fragment thereof. Therefore, Applicants maintain that the issues with respect to the manipulation of endogenous neural precursors in general are not relevant to the claimed methods reciting specific steps for differentiating cells *in vitro* or *in vivo*.

Referring to Mehler et al., Arch. Neurol. 56:780-784 (1999), Applicants respectfully maintain that the issues with respect to environmental cues for differentiation of neural progenitor cells that may or may not be present in normal or neuropathological conditions are not relevant to the claimed methods. In particular, the claims recite the steps of contacting the progenitor cells with a differentiating agent and introducing into the progenitor cells a nucleic acid molecule encoding an active MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death. Thus, the claims recite specific steps for differentiating cells *in vitro* or *in vivo*.

Applicants respectfully maintain that the issues discussed in the Office Action regarding Jackowski, Br. J. Neurosurg. 9:303-317 (1995), Grados-Munro et al., J. Neurosci. Res. 74:479-485 (2003), and Filbin, Nat. Rev. 4:1-11 (2003), are not relevant to the claimed methods. The claimed methods are directed to differentiating progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death by contacting the progenitor cells with a differentiating agent and introducing a nucleic acid encoding an active MEF2 polypeptide or active fragment thereof into the progenitor cells, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apopototic cell death. With respect to the comment in the Office Action in the paragraph bridging pages 13-14 that no support has been provided for the assertion that these references are not relevant, Applicants respectfully maintain the position of record. In particular, Jackowski is a review article that discusses neural injury repair and "outlines possible therapeutic approaches that may enable

more effective CNS regeneration to be accomplished in the future" (abstract; emphasis added). Similarly, Applicants respectfully submit that both Grados-Munro et al. and Filbin are, at best, review articles describing myelin-associated inhibitors of axon regeneration. Applicants respectfully maintain, for the reasons of record, that the claimed methods are directed to differentiating progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death by contacting the progenitor cells with a differentiating agent and introducing a nucleic acid encoding a MEF2 polypeptide or active fragment thereof into the progenitor cells.

Applicants respectfully maintain that Cheng et al., Blood 92:83-92 (1998), previously provided in the response filed September 3, 2004, provides corroborative evidence that one skilled in the art would have been able to introduce a nucleic acid molecule into human progenitor cells. In particular, Applicants respectfully maintain that the optimized retroviral gene-transfer protocol described by Cheng et al. is relevant to *in vivo* uses since such viral vectors are routinely used for *in vivo* transduction. Furthermore, Applicants respectfully maintain that Hanazono et al., Stem Cells 19:12-23 (2001), supports Applicants contention that viral vectors were routinely used for gene transfer into hematopoietic stem cells. Applicants respectfully disagree with the assertion in the Office Action on page 14 that Applicants' contention is not accurate. The Office Action quotes from Hanazono et al., "[t]hese vectors are being examined *in vivo* in nonhuman primate models," referring to page 16, column 2, paragraph 1, as support that Applicants' contention that viral vectors were routinely used for gene transfer into hematopoietic stem cells is not accurate. Applicants respectfully disagree, noting that Hanazono et al. describes the use of retroviral vectors and lentiviral vectors (page 15, right column, to page 16, left column). With regard to the quote from Hanazono et al., this quote is in the following context:

> In an attempt to improve expression, retroviral vectors derived from the myeloproliferative sarcoma virus (MPSV), murine stem cell virus (MSCV), and spleen focus-forming virus have recently been developed [86-89]. In general, these vectors differ from the Moloney murine leukemia virus (MoMLV) vectors in the LTR promoter-enhancer region and the primer-binding site. These vectors have been shown to result in improved expression of transgenes compared to MoMLV vectors in vivo in mice, and in vitro in human primary hematopoietic cells. These vectors are being examined in vivo in nonhuman primate models. [page 16, right column, first paragraph]

Thus, Applicants note that the quote describing "[t]hese vectors" is referring to retroviral vectors derived from MPSV, MSCV and spleen focus-forming virus. Furthermore, Applicants respectfully maintain that, not only does Hanazono et al. support Applicants' position that viral vectors were routinely used for gene transfer into hematopoietic stem cells (page 15, right column, to page 16, left column), but Cheng et al. as well, as discussed previously, also corroborates the routine use of viral vectors to transduce human hematopoietic stem cells.

Applicants further maintain that Zwaka and Thomson, Nat. Biotechnol. 21:319-321 (2003), which was previously submitted with the response filed September 3, 2004, corroborates Applicants' position that electroporation can be used to produce stably transfected human ES cells. In the paragraph in the Office Action bridging pages 14-15, it is asserted that the specification does not provide specific guidance for using electroporation techniques for transfecting human ES cells and that "only a laundry list" of techniques that could be used to introduce a nucleic acid molecule into an embryonic stem cell is provided. To the contrary, Applicants respectfully maintain that the specification teaches exemplary well known methods for introducing a MEF2 polypeptide into a progenitor cell such as an embryonic stem cell, including exemplary references teaching such well known techniques (see specification, for example, on page 54, line 4, to page 55, line 17). Applicants respectfully maintain that, based on the teachings in the specification and what was well known to those skilled in the art, one skilled in the art would have been enabled to make and use the methods as claimed.

Regarding Eiges et al., Curr. Biol. 11:514-518 (2001), which was submitted in the response filed September 3, 2004, Applicants respectfully maintain that this reference corroborates Applicants' position that routine transfection methods, including electroporation, could be used to transfect human ES cells. Furthermore, Eiges et al. teaches that other routine transfection methods such as Fugene and ExGen 500 could also be used to successfully transfect human ES cells. As discussed in the previous responses, Applicants respectfully maintain that transfection methods such as Fugene and ExGen 500 were routine and available to one skilled in the art at the time of filing of the priority application, June 5, 2000, as corroborated by Ferrari et al., Gene Therapy 4:1100-1106 (1997), and Uyttersprot et al., Mol. Cell. Endocrin. 142:35-39 (1998). These references were provided to corroborate that the transfection methods for human ES cells described in Eiges et al. were well known and available to one skilled in the art.

Applicants respectfully maintain that Eiges et al. describes the successful transfection of human ES cells using routine methods well known to those skilled in the art at the time of filing of the priority application and that it would have been routine for one skilled in the art to try various known transfection methods to successfully transfect human ES cells, as corroborated by Eiges et al. Moreover, Applicants respectfully disagree with the assertion in the Office Action on page 15 that the specification does not provide specific guidance for transfecting human ES cells. In particular, the specification teaches numerous exemplary methods for introducing a nucleic acid molecule into a progenitor cell, including electroporation and viral vectors (page 54, line 4, to page 55, line 17). Thus, contrary to the assertion in the Office Action, Applicants respectfully maintain that the specification teaches specific guidance for introducing a nucleic acid into a progenitor cell, including human ES cells.

Applicants respectfully maintain, as discussed in previous responses, that the claimed methods of differentiating progenitor cells do not recite, nor do they require, a particularly "high efficiency" of transfection. Rather, one skilled in the art understands that cell populations stably expressing an introduced nucleic acid molecule can be routinely prepared using, for example, standard methods such as antibiotic selection in order to select for a transfected population of cells. Thus, the skilled person understands that, even if progenitor cells were not transfected with particularly high efficiency, one skilled in the art would have been able to produce a population of progenitor cells predominantly or uniformly containing a MEF2 polypeptide using only routine methods.

Regarding the discussion on page 18 of the Office Action relating to Milward et al., J. Neurosci. Res. 50:862-871 (1997), Applicants previously discussed Milward et al. as corroborating that differentiation can occur *in vivo* in injury and disease. In the Office Action dated August 12, 2005, it was asserted that the references by McDonald et al., Nat. Med. 5:1410-1412 (1999), and Liu et al., Proc. Natl. Acad. Sci. USA 97:6126-6131 (2000), described "an injury model" rather than "an ongoing pathological process." Milward et al. was discussed previously on the record as exemplifying an "ongoing pathological process" as distinct from "an injury model." The Office Action maintains that the disease model described by Milward et al. is a genetic defect that is not considered to be "an ongoing pathological process." Regardless of how the disease model of Milward et al. is viewed, Applicants respectfully maintain that it is

clearly exemplary of disease model that is not "an injury model" and supports Applicants' position of record.

Applicants respectfully maintain, for the reasons of record, that the specification provides sufficient description and guidance to enable the claimed methods. In further corroboration, submitted herewith as Exhibit 1 is a Rule 132 Declaration executed by Dr. Stuart Lipton, one of the inventors, along with related Exhibits A and B. Exhibit A was recently published in The Journal of Neuroscience (J. Neurosci. 28:6557-6568 (2008)), describing the neurogenic and antiapoptotic effect of expression of MEF2C in murine embryonic stem cells. Exhibit A further describes that MEF2C-expressing neuronal progenitor cells transplanted into a mouse model of cerebral ischemia can successfully differentiate into functioning neurons and ameliorate stroke-induced behavioral deficits. Exhibit B provides data showing the neurogenic activity of MEF2C in human embryonic stem cells. It is respectfully submitted that the evidence provided in Exhibits 1, A and B submitted herewith corroborates Applicants' position that the specification provides sufficient description and guidance to enable the claimed methods.

For the reasons of record and as discussed above, and further in view of the corroborative evidence submitted previously and herewith, Applicants respectfully maintain that the specification provides sufficient description and guidance to enable the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Rejection Under 35 U.S.C. § 112, Second Paragraph

The rejection of claims 20 and 78 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite is respectfully traversed. Applicants respectfully maintain that the claimed methods are clear and definite. Nevertheless, claims 20 and 78 have been amended to recite "neuronal cells protected from apoptotic cell death" to more closely parallel the language of base claims 1 and 59, respectively, and to provide clear antecedent basis. Applicants respectfully submit that the claims are clear to one skilled in the art. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Rejections Under 35 U.S.C. § 102

The rejection of claims 1-4, 18, 58-62, 76 and 79 under 35 U.S.C. § 102(a) as allegedly anticipated by Okamoto et al., Proc. Natl. Acad. Sci. USA 97:7561-7566 (2000), is respectfully traversed. Applicants respectfully maintain that the claimed methods are novel over Okamoto et al. Applicants point out that the priority application, serial No. 60/209,539, was filed June 5, 2000. As corroborated by the evidence submitted as Exhibit B in the previous response, Okamoto et al. was published online on June 13, 2000. Applicants further point out that the subject matter of Okamoto et al. was substantially disclosed in priority provisional application 60/209,539. For example, Figures 6-10 of the priority provisional application correspond to Figures 1-5 of Okamoto et al. Applicants respectfully submit that the priority date of the subject application is prior to the publication date of Okamoto et al. Therefore, Applicants respectfully submit that Okamoto et al. is not proper prior art. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The rejection of claims 1, 2, 18, 58-60, 76 and 79 under 35 U.S.C. § 102(b) as allegedly anticipated by Krainc et al., J. Biol. Chem. 273:26218-26224 (1998), is respectfully traversed. Applicants respectfully maintain that the claimed methods are novel over Krainc et al.

In the Office Action on page 22, Krainc et al. is described as disclosing that the plasmid pG/DNA, containing the N-terminal DNA binding domain of MEF2C, was stably transected into P19 cells, referring to Figure 5 and page 26222, column 2, paragraph 2. The referenced paragraph from Krainc et al. reads as follows:

> To gain more direct evidence that endogenous MEF2C is involved in *NR1* gene expression *in vivo*, we monitored NR1 mRNA levels during neuronal differentiation in the presence or absence of a dominant-negative MEF2C protein. We stably transfected the plasmid pG/DN, which contains the cDNA sequence of the $NH_2$-terminal DNA binding domain of MEF2C, into P19 cells. These cells differentiate into a neuronal phenotype after treatment with 13-*cis*-retinoic acid, and then express MEF2C (55) as well as glutamate receptor mRNAs (38). By reverse transcriptase-PCR, we observed induction of NR1 mRNA expression in P19 cells after differentiation with 13-*cis*-retinoic acid for 7 days (Fig. 5A, lane 4). This induction was totally abolished, however, in p19 cells stably expressing the dominant-negative MEF2C (Fig. 5A, lane 5). [emphasis added]

As described in the passage above and on page 26219, column 1, under "Stable Transfection," the pGK/DN construct contains a dominant-negative MEF2C cDNA. In contrast, the claimed methods include the step of introducing into the progenitor cells a nucleic acid molecule encoding an active MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death. Krainc et al. provides no teaching of introducing into progenitor cells a nucleic acid molecule encoding an active MEF2 polypeptide or an active fragment thereof to produce a cell population containing neuronal cells protected from apoptotic cell death. Absent such a teaching, Applicants respectfully submit that Krainc et al. cannot anticipate the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The rejection of claims 1, 2, 18, 58-60, 76 and 79 under 35 U.S.C. § 102(b) as allegedly anticipated by Skerjanc et al., FEBS Lett. 472:53-63 (2000)(hereinafter Skerjanc et al., 2000), as evidenced by Skerjanc et al., J. Biol. Chem. 273:34904-34910 (1998)(hereinafter Skerjanc et al., 1998), is respectfully traversed. Applicants respectfully maintain that the claimed methods are novel over Skerjanc et al., 2000.

Applicants respectfully maintain that Skerjanc et al., 2000, does not teach the claimed methods. As discussed in the previous response, Skerjanc et al., 2000, indicates that "P19 cells differentiate into neuroectodermal lineages when aggregated with retinoic acid but not when aggregated with $ME_2SO$ [DMSO] or in the absence of retinoic acid" (page 54, left column, first sentence under "Results and discussion"). Thus, Applicants maintain the position of record with respect to the description in Skerjanc et al., 2000, in that DMSO does not cause P19 cells to differentiate into neuroectodermal lineages, in contrast to retinoic acid. With respect to the comments in the Office Action on page 24 regarding the indication in the Skerjanc et al., 2000, abstract and on page 53, column 2, second paragraph, relating to nuerogenesis induced by MEF2C, it is respectfully pointed out that Table 1 (page 54) of Skerjanc et al., 2000, as did Skerjanc et al., 1998, shows that expression of MEF2C, in the absence of DMSO, differentiates P19 cells into cardiac cells. In the results and discussion section (page 56, left column, last paragraph), Skerjanc et al., 2000, states "[I]n summary, MEF2C initiates neurogenesis in P19 cells aggregated with $ME_2SO$." Thus, Applicants maintain the position of record with respect to

the characterization of Skerjanc et al., 2000, that is, its description of the induction of neurogenesis in cells expressing MEF2C and aggregated with DMSO.

As discussed in the previous response, the specification teaches that a "differentiating agent" is a naturally occurring or synthetic cytokine, growth factor or other compound that causes or enhances a progenitor cell to have one or more characteristics of a neuronal cell (page 46, lines 18-22). Exemplary differentiating agents include retinoic acid, neurotrophic factor 3, epidermal growth factor, insulin-like growth factor-1, and platelet derived growth factor. Moreover, Skerjanc et al., 2000, does not teach the claimed methods, which include contacting the progenitor cells with a differentiating agent and introducing into the progenitor cells a nucleic acid molecule encoding an active MEF2 polypeptide or an active fragment thereof, thereby differentiating the progenitor cells to produce a cell population containing neuronal cells protected from apoptotic cell death. Absent such a teaching, Applicants respectfully submit that Skerjanc et al., 2000, cannot anticipate the claimed methods. Accordingly, Applicants respectfully request that this rejection be withdrawn.

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned agent if there are any questions.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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